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PIK3CA mutations are associated with reduced pathological complete response rates in primary HER2-positive breast cancer: pooled analysis of 967 patients from five prospective trials investigating lapatinib and trastuzumab†

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Background: The predictive value of *PIK3CA* mutations in HER2 positive (HER2+) breast cancer treated with neoadjuvant anti-HER2 and chemotherapy has been reported, but the power for subgroup analyses was lacking.
Patients and methods: We combined individual patient data from five clinical trials evaluating *PIK3CA* mutations and associations with pathological complete response (pCR), disease-free survival (DFS) and overall survival (OS). Patients

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received either trastuzumab (T), lapatinib (L) or the combination T/L in addition to a taxane-based chemotherapy. *PIK3CA* was genotyped in tumour biopsies taken before therapy.

Results: A total of 967 patients were included in this analysis; the median follow-up is 47 months. Overall, the pCR rate was significantly lower in the *PIK3CA* mutant compared with the wild-type group (16.2% versus 29.6%; $P < 0.001$). Within the hormone-receptor positive (HR+) subgroup, the *PIK3CA* mutant group had a pCR rate of only 7.6% compared with 24.2% in the wild-type group ($P < 0.001$). In contrast, in the HER2+/HR– group, there was no difference in pCR (27.2% versus 36.4%; $P = 0.125$) according to *PIK3CA* mutation status (interaction test $P = 0.036$). According to treatment arm, the pCR rate for mutant versus wild-type was 20.3% versus 27.1% for T ($P = 0.343$), 11.3% versus 16.9% for L ($P = 0.369$) and 16.7% versus 39.1% for T/L ($P < 0.001$). In the HR+ T/L group, the pCR rate was 5.5% versus 33.9% (interaction between HR and *PIK3CA* genotype $P = 0.008$). DFS and OS were not significantly different by mutation status, though the incidence rate of events was low. However, HR+/*PIK3CA* mutant patients seemed to have significantly worse DFS {hazard ratio (HR) 1.56 [95% confidence interval (CI) 1.00–2.45], $P = 0.050$; $P_{interaction} = 0.021$}. T/L tended to improve DFS compared with T in the wild-type cohort, especially in the HR– group [HR 0.72, 95% CI (0.41–1.25), $P = 0.242$].

Conclusion: Overall *PIK3CA* mutant/HER2+ tumours had significantly lower pCR rates compared with wild-type tumours, however mainly confined to the HR+/*PIK3CA* mutant population. No definite conclusions can be drawn regarding survival.

Key words: *PIK3CA*, HER2+ breast cancer, double anti-HER2 treatment, pathological complete response, neoadjuvant, survival

introduction

In HER2-positive (HER2+) primary breast cancer (BC), pathological complete response (pCR) using neoadjuvant therapy continuously increased by different strategies: addition of trastuzumab to chemotherapy, increased duration of chemotherapy or double anti-HER2 therapy [1]. In studies using up to 24 weeks of an anthracycline- and taxane-based chemotherapy and using a double blockade, the pCR rate increased to more than 60%, approaching 75% in HER2+/hormone-receptor negative (HR−) breast cancer [2]. The only predictive marker for anti-HER2 treatment remains HER2, but resistance markers are lacking. One mechanism of interest for HER2 is the Phosphatidylinositol 3-kinase (PIK3) pathway. *PIK3CA* and its counterpart Phosphatase and tensine homologue (PTEN) have long been investigated. While PTEN status is difficult to be measured, mutations of the *PIK3CA* are common in BC and can reliably be assessed by different methods [3]. These mutations are predominantly found in hotspots located in the helical and kinase domains (exons 9 and 20), resulting in activation of the kinase. It could recently be demonstrated that *PIK3CA* mutations predict lower pCR to double blockade with trastuzumab and lapatinib as well as trastuzumab plus afatinib in HER2+ primary BC [4–7].

Despite the fact that data on *PIK3CA* mutations demonstrate significantly lower pCR rates, this has not led to an inclusion of the marker into routine diagnostics.

materials and methods

end points

The primary aim of this study was to investigate the association of pCR and *PIK3CA* mutations in primary HER2+ BC treated with one or two HER2 targeting agents.

Secondary aims were to determine the association of pCR and *PIK3CA* mutations using different pCR definitions, different subgroups defined by exon (exon 9 versus exon 20), HR status (HR+ versus HR−) and anti-HER2 treatment (trastuzumab versus lapatinib versus trastuzumab plus lapatinib) and to test how these factors influenced disease-free survival (DFS) and overall survival (OS) overall.

pCR was defined as no invasive and no non-invasive residuals in breast and lymph nodes (ypT0 ypN0). Patients with missing pCR assessment or information were regarded as no pCR.

DFS was defined as time in months from randomization to (local or distant) disease recurrence, secondary malignancy or death due to any cause. OS was defined as time in months from randomization to death due to any cause.

patients and treatment

PIK3CA mutation was assessed in pre-treatment tumour samples from patients enrolled in five prospectively randomized neoadjuvant trials investigating the dual blockade with T/L (supplementary Figure S1, available at *Annals of Oncology* online). In addition to taxane-based chemotherapy, patients received either trastuzumab, lapatinib or the combination of trastuzumab and lapatinib. Detailed information on the design and eligibility criteria of the included studies can be accessed elsewhere (GeparQuattro [8], GeparQuinto [9], GeparSixto [10], NeoALTTO [11], CHERLOB [12]).

For GeparQuattro and GeparQuinto, the central HER2 status (IHC3+ or ISH ratio >2.2) was determined retrospectively, and only centrally HER2-positive tumours were included in the *PIK3CA* mutation analysis. In GeparSixto, HER2 status was assessed centrally before randomization. In the NeoALTTO study, HER2 was assessed centrally or locally (after central laboratory accreditation) and for the CHERLOB study, central HER2 testing was available.

In the GeparSixto study, *PIK3CA* mutations were assessed prospectively during the recruitment phase of the study in all patients. For all other studies, *PIK3CA* mutations were assessed retrospectively using formalin-fixed, paraffin-embedded (FFPE) core biopsies that had been prospectively collected.

In the CHERLOB study, in 10 patients, *PIK3CA* was assessed on the surgical tissue.

All patients provided their written informed consent for study participation, biomaterial collection and use of biomaterial in research projects. The relevant authorities and ethics committees approved the studies. The REMARK (Reporting Recommendations for Tumour Marker Prognostic Studies) criteria were followed [13].

PIK3CA mutation assessment

PIK3CA mutations were evaluated in FFPE or fresh-frozen tumour material with a tumour content of ≥20% for the Geparstudies, ≥10% for the

NeoALTTO and ≥50% for the CHERLOB samples. *PIK3CA* was genotyped using Sanger sequencing (Geparstudies), Sequenom mass-spectrometry genotyping (NeoALTTO) or pyrosequencing (CHERLOB). Detailed method descriptions can be found elsewhere [5–7, 14].

Various methods were used to determine *PIK3CA* mutation status. In 504 samples, Sanger sequencing evaluated hotspot mutations in exon 9, encoding the helical domain of *PIK3CA* (E542K, E545K, E547K, A533V, A533T) and in 20, encoding the catalytic domain (H1047R, H1047L, G1007S) [15]. In NeoALTTO ($n = 355$), the Sequenom mass-spectrometry genotyping system was used to test for hotspot mutations E542A/K, E545A/K and H1047R/L for *PIK3CA* [6]. In 108 samples, pyrosequencing was used. The approach permitted the identification of mutations in codons 542, 545 and 546 of exon 9 (E542K, E545K, E545A, E545G, Q546E, Q546K) and codons 1043, 1047 and 1049 of exon 20 (M1043I, H1047Y, H1047R, H1047L, G1049R, G1049S) of the *PIK3CA* gene [7].

statistical analysis

Associations between mutation status (wild-type versus mutant), clinicopathologic characteristics and pCR were investigated with a (continuity corrected) χ^2 tests for categorical variables. Univariate analysis using a binary logistic regression model was carried out to estimate the magnitude of the effect. Odds ratios (ORs) and 95% confidence intervals (CIs) with a two-sided Wald *P* value are given. A multivariable Cox regression model was fit to adjust for known baseline characteristics (age, tumour stage, nodal stage, histological type, grading, HR status, treatment and study). A Breslow day interaction test was carried out for certain (binary) subgroup analyses; an interaction term in logistic regression was used to test for interaction of subgroup factors with more than two categories. Survival analyses were carried out using the Kaplan–Meier product-limit method and the Cox proportional hazards regression models to test the prognostic value of *PIK3CA* mutation status [hazard ratios (HRs) and 95% CIs] for DFS and OS. An interaction term in the Cox proportional hazard model was used to test for interaction for time-to-event end points. All *P* values are two-sided, with a *P* value of ≤5% considered to be statistically significant. No adjustment for multiple testing was carried out. Statistical analysis was conducted using SAS version 9.2 under SAS Enterprise Guide 4.3 (SAS Institute Inc., Cary, NC).

results

baseline characteristics and mutation frequencies

In the overall study cohort ($N = 967$), the *PIK3CA* mutation rate was 21.7%, 21.4% in the GeparStudies, 22.5% in NeoALTTO and 20.4% in the CHERLOB study (supplementary Table S1, available at *Annals of Oncology* online). Overall, the mutations in exon 20 were twice as high as in exon 9 (14.5% versus 7.2%). Similar rates were seen in the different studies (Geparstudies 13.1% versus 8.3%, NeoALTTO 16.3% versus 6.2%, CHERLOB 14.8% versus 5.6%). There was no difference in the *PIK3CA* mutation rate by HR status (supplementary Table S2, available at *Annals of Oncology* online).

correlation of mutation and pCR

Overall, pCR rates were significantly lower in the *PIK3CA* mutant cohort (16.2%), compared with one in the wild-type cohort [29.6% (OR 0.460; 95% CI 0.308–0.685], $P < 0.001$]. This difference was mainly confined to the HR+ subgroup, with a significant interaction between *PIK3CA* and HR status (Figure 1 and supplementary Table S3, available at *Annals of Oncology* online). A significantly different pCR rate between *PIK3CA* mutant and wild-type tumours could only be observed in the group receiving trastuzumab and lapatinib [16.7% versus 39.1%; OR 0.311 (95% CI 0.168–0.577), $P < 0.001$]; however, there was no significant interaction of the *PIK3CA* mutation status with type of anti-HER2 treatment ($P = 0.189$) (Figure 1). When taking treatment and HR status, a significant interaction of *PIK3CA* mutation with the HR status was confined to the cohort with trastuzumab and lapatinib (supplementary Figure S2, available at *Annals of Oncology* online). After adjustment for age, tumour stage, nodal status, histological tumour type and grading, *PIK3CA* mutation status, HR status and treatment provided independent predictive information for achieving a pCR (supplementary Figure S3, available at *Annals of Oncology* online). Treatment was only a significant independent predictor for pCR in the cohort with *PIK3CA* mutation

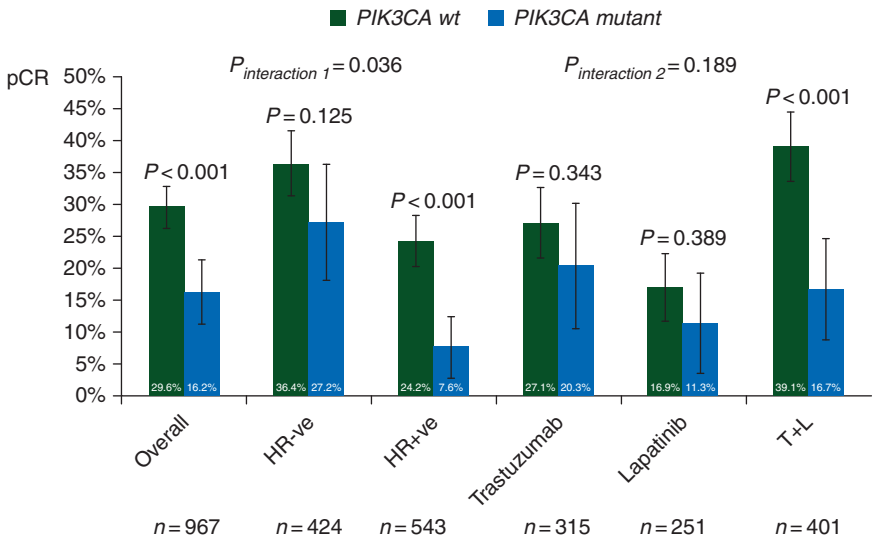

Figure 1. Pathological complete response rates according to *PIK3CA* mutation status overall, by HR status and anti-HER2 treatment.

(supplementary Table S3 and supplementary Figure S4 available at *Annals of Oncology* online).

survival analysis

The median follow-up was 47 months: 40 months for Geparstudies (68 months for GeparQuattro, 54 months for GeparQuinto and 31 months for GeparSixto), 66 months for the NeoALTTO study and 46 months for the CHERLOB study. There was no statistically significant difference in DFS (Figure 2A) (HR mutant versus wild-type 1.07; 95% CI 0.76–1.50; $P = 0.691$) and OS (supplementary Figure S5, available at *Annals of Oncology* online).

However, when taking into consideration HR status, there was a significant interaction between HR status and *PIK3CA* genotype. The HR+ cohort showed a statistically significant worse DFS for the mutant cohort [Figure 2C; HR 1.56 (95% CI 1.00–2.45), $P = 0.050$], while there was a non-significant trend for a better DFS of the *PIK3CA* mutant /HR− group [Figure 2B; HR 0.69 (95% CI 0.41–1.17), $P = 0.170$] with a statistically significant interaction test ($P = 0.021$).

Since pCR is a known prognostic factor in HER2+ BC, combined survival analyses by pCR and *PIK3CA* and HR status were carried out (Figure 2D). In the pCR group, patients with a *PIK3CA* mutation tended to have better survival than those in the wild-type group irrespective of the HR status (log-rank test mutant versus wild-type $P = 0.149$). Overall, there was no difference in DFS by mutation status in the non-pCR cohort ($P = 0.797$). But HR non-pCR patients had a better survival with a mutation [HR mut versus wt HR1.53 (95% CI 0.96–2.43) $P = 0.073$], whereas HR+ non-pCR patients had a better survival without a mutation [HR mut versus wt HR 0.68 (95%CI 0.39–1.17), $P = 0.164$] (supplementary Figure S6A and B, available at *Annals of Oncology* online).

An explorative analysis whether a long-term treatment effect was detectable within the *PIK3CA* wild-type compared with the mutant cohort suggested, although not statistically significantly different, that, similar to the pCR results, dual anti-HER2 treatment improves DFS only in the wild-type cohort but not in the mutant cohort (log rank $P_{interaction}$=0.392) and only in the HR population [HR 0.72 (95% CI 0.41–1.25), $P = 0.242$] (Figure 2E and F).

discussion

We confirmed in almost 1000 HER2+ primary BC that tumours harbouring a *PIK3CA* mutation have a significantly lower pCR rate, especially in the group receiving T/L, although a trend within the monotherapy anti-HER2 treatment groups could be seen. Combining both groups into a monotherapy anti-HER2 cohort ($n = 566$ patients) demonstrated a strong trend towards a lower pCR rate in the mutant compared with the wild-type cohort (15.9% versus 22.7%; $P = 0.125$), suggesting a small effect for *PIK3CA* mutations also there. To show an absolute 7% pCR difference to be of statistical significance, a sample size of several thousands would have been needed. However, in a recent smaller study, PTEN and the PIK3 downstream marker pEBP4 added information to the *PIK3CA* mutation status in a trastuzumab cohort [16].

Interestingly, *PIK3CA* status was observed to have a significant interaction with the HR status in the group treated with trastuzumab and lapatinib.

The role of *PIK3CA* in assessing the predictive effect in neoadjuvant therapy using the double blockade with trastuzumab plus pertuzumab has been less thoroughly investigated showing results comparable to ours [17, 18].

The Cleopatra study reported that patients with a *PIK3CA* mutation in their primary tumor had a significantly worse PFS, but mutation status did not predict greater benefit from double blockade [19, 20].

In the DFS analysis, as in the pCR analysis, the HR status interacted with the *PIK3CA* mutation status. HR− patients had a better DFS if their tumour harboured a *PIK3CA* mutation, whereas in HR+ patients, DFS was worse.

Despite a significant difference in the pCR rate, there was no difference in DFS and OS for patients with tumours harbouring a *PIK3CA* mutation, which is consistent with the data from the NSABP-B31 trial, even though the FinHER trial showed an improved survival within the first 3 years for the *PIK3CA* mutant cohort [21, 22]. The data for *PIK3CA* are not very conclusive regarding survival. Numbers were either too small, or only a subcohort of the trial cohort was investigated or, as in the neoadjuvant setting, patients received longer anti-HER2 treatment in some trials or switched from lapatinib to trastuzumab after surgery [23]. Although exploratory, there seems to be benefit from the T/L combination in *PIK3CA* wt HR−/HER2+ patients. The non-pCR group of that cohort had the worst prognosis from all subgroups. A confirmation of these data in the much larger ALTTO trial would support the role of T/L in HR−/HER2+/*PIK3CA* wt. However in the HR+ group, the non-pCR *PIK3CA* mutant cohort had the worst prognosis, which warrants a chemofree quadruplet of dual blockade with anti-hormone-therapy and a more specific PI3K to be further investigated.

In the BOLERO 1 and 3 studies conducted in HER2+ metastatic BC, the effect of everolimus, an mTOR inhibitor acting downstream in the PI3Kinase pathway, was only detectable in the cohort harbouring a *PIK3CA* mutation [24]. Similar results were seen in Belle-2, when the pan PI3K inhibitor buparlisib in addition to fulvestrant in HER2−/HR+ metastatic BC improved PFS only in the group with a *PIK3CA* mutation detected in circulating tumour DNA [25]. The NeoPHOEBE study investigating buparlisib in HER2+ BC is too small and included only eight patients with a *PIK3CA* mutation, but demonstrated an increased response rate after 6 weeks by adding buparlisib to trastuzumab in the HR+ cohort [26].

This analysis has several strengths and limitations. The integrated analysis using data from three different projects, including five different clinical trials, has the power to demonstrate a positive interaction of *PIK3CA* mutation status and the HR status regarding pCR when trastuzumab and lapatinib are used. However, the power for the survival analysis is still limited. To account for the fact that data from three different studies were combined, the multivariable models were adjusted for 'study'. The different studies have used different sequencing approaches. Sanger sequencing has been used as a standard method for a long time, while Sequenom and pyrosequencing technologies have been introduced more recently. The backgrounds of the methods are different; in particular, the Sequenom technology

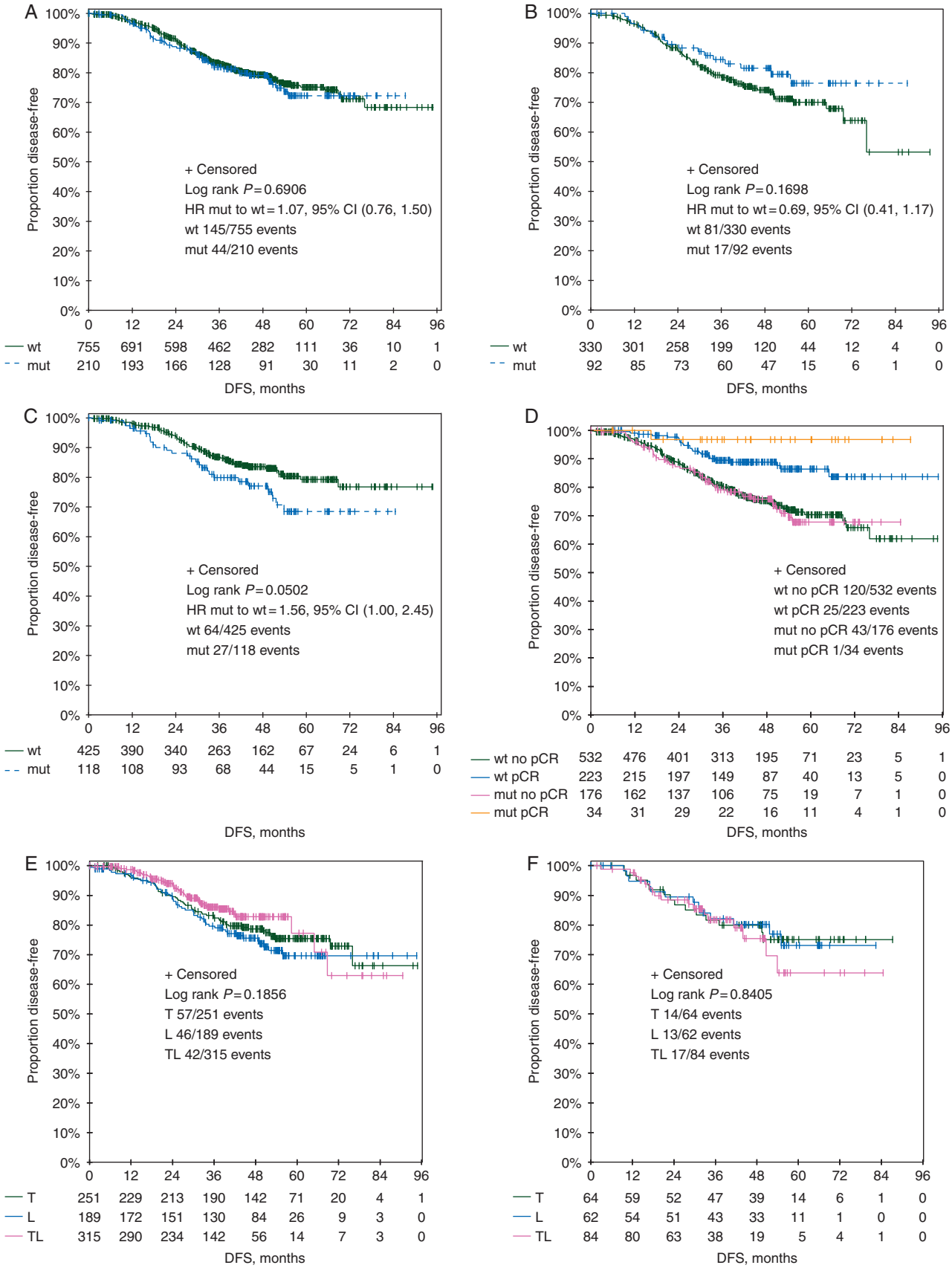

Figure 2. Survival curves: (A) disease-free survival by *PIK3CA* mutation status; (B and C) disease-free survival by *PIK3CA* mutation status in hormone receptor negative (B) and positive (C) tumours (interaction of HR status and *PIK3CA* $p = 0.021$). (D) Disease-free survival according to pathological complete response and *PIK3CA* mutation status. Disease-free survival by anti-HER2 treatment (trastuzumab, lapatinib and trastuzumab plus lapatinib) in patients with *PIK3CA* wild-type (E) and *PIK3CA* mutant (F) tumours.

uses detection of DNA fragments by MALDI-TOF mass spectrometry. All three approaches, however, are focused on the analysis of defined mutational alterations. This makes them particularly suitable for the analysis of *PIK3CA*, which has mutations located at defined hotspots. Consequently, all three methods are well established and the *PIK3CA* mutation rates in the different studies are within the same range. Comparative studies have been carried out for comparison of Sequenom analysis versus targeted next-generation sequencing (NGS) and Sanger sequencing versus targeted NGS with comparable results by both methods [27, 28].

In conclusion, *PIK3CA* mutation is associated with a lower pCR rate overall and particularly in HER2+ primary BC treated with trastuzumab and lapatinib in addition to chemotherapy. This pooled analysis also demonstrated a significant interaction between HR and *PIK3CA* status and treatment for both pCR and DFS end points. The data could be used to further subdivide the HER2+ population in order to improve outcome.

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disclosure

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Pooled analyses of eribulin in metastatic breast cancer patients with at least one prior chemotherapy

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Background: Based on data from two multicenter, phase III clinical trials (Studies 301 and 305), eribulin (a microtubule dynamics inhibitor) is indicated in the European Union (EU) for patients with locally advanced or metastatic breast cancer (MBC) after ≥1 prior chemotherapy for advanced disease, including an anthracycline and a taxane in either the adjuvant or metastatic setting. Data from Studies 305 and 301 were pooled to investigate the efficacy of eribulin in various subgroups of patients who matched the EU label, including those with human epidermal growth factor receptor 2 (HER2)-negative and triple-negative disease.

Patients and methods: In Study 305 (NCT00388726), patients were randomized 2:1 to eribulin mesylate 1.4 mg/m^2 (equivalent to eribulin 1.23 mg/m^2 [expressed as free base]) intravenously on days 1 and 8 every 21 days] or treatment of physician's choice after 2–5 prior chemotherapies (≥2 for advanced disease), including an anthracycline and a taxane (in early/advanced setting). In Study 301 (NCT00337103), patients were randomized 1:1 to eribulin (as above) or capecitabine (1.25 g/m^2 orally twice daily on days 1–14 every 21 days) following ≤3 prior chemotherapies (≤2 for advanced disease), including an anthracycline and a taxane. Efficacy end points were investigated in the intent-to-treat population and subgroups, pooled as discussed above.

Results: Overall, 1644 patients were included (eribulin: 946; control: 698); baseline characteristics were well matched. Overall survival was significantly longer with eribulin versus control ($P < 0.01$), as were progression-free survival and clinical benefit rate (both $P < 0.05$). Significant survival benefits with eribulin versus control were observed in a wide range of patient subgroups, including HER2-negative or triple-negative disease (all $P < 0.05$).

Conclusion: Our findings underline the survival benefit achieved by eribulin used according to EU label in the overall MBC population and in various subgroups of interest, including patients with HER2-negative and triple-negative disease.

Key words: metastatic breast cancer, triple-negative breast cancer, eribulin, clinical trial, survival, pooled analysis

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